The Pending Claims

Prior to entry of the above amendments, Claims 1-18 are pending. Claims 1-5 are directed to oligonucleotide primers for simultaneous amplification of multiple target DNA sequences under a single set of reaction conditions in a multiplex polymerase chain reaction. Claim 6 is directed to a method of simultaneous amplification of multiple DNA target sequences present in a DNA sample. Claims 7-9 are directed to a method for simultaneously detecting the presence of multiple defined target DNA sequences in a DNA sample. Claims 10-12 are directed to a method for high-throughput genetic screening to simultaneously detect the presence of multiple defined target sequences in DNA samples obtained from one or more individuals. Claim 13 is directed to a method for simultaneously detecting multiple defined target sequences in a DNA sample. Claims 14-16 are directed to a method of screening to simultaneously amplify and detect multiple target sequences of interest in DNA. Claims 17 and 18 are directed to a plurality of amplified target sequences of interest amplified and detected according to the methods of Claims 13 and 14, respectively.

The Office Action

The objection to the declaration in the Office Action mailed October 9, 1996 has been withdrawn in light of the amendment of the response filed February 10, 1997.

The objection to disclosure in paragraph 1 of the Office Action mailed October 9, 1996 has been withdrawn in light of the amendments of the response filed February 10, 1997.

The objections to Claims 2, 5-7 and 10 in paragraph 2 of the Office Action mailed October 9, 1996 have been withdrawn in light of the amendments of the response filed February 10, 1997.

The rejection of Claims 4 and 12, under 35 U.S.C. 112, second paragraph, in paragraph 3 of the Office Action mailed October 9, 1996, has been withdrawn in light of the amendments of the response filed February 10, 1997.

The rejection of Claims 1-12, under 35 U.S.C. 103, made in paragraphs 4-6 of the Office Action mailed October 9, 1996, has been maintained and the rejection has now been applied over new Claims 13-18.

The amendment filed February 10, 1997 has been objected to under 35 U.S.C. 132, because it purportedly introduces new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows; "a plurality of amplified target sequences of interest." Support for this addition purportedly cannot be found in the specification, and Applicant has been required to cancel the new matter.

Claims 17 and 18 have been rejected under U. S. C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. A plurality of amplified target sequences of interest was not described in the specification.

Claims 17 and 18 have been rejected under 35 U.S.C. 112, second paragraph, because of the language "a plurality of amplified target sequences of interests". It cannot be determined from the specification what is encompassed by the plurality of nucleic acids defined only by how nucleic acids are detected.

No claims are allowable over the prior art, and because Applicant's amendment necessitated the new ground(s) of rejection, the Examiner's action has been made final.

Amendments

The amendment at page 6, line 13 to change "II" to --H--, corrects a typographical error. Support is found in Figure 5, top line, above lanes 10-11.

Applicant has amended Claim 1. Support for the amendment is found, for example, at page 8, lines 3-22.

Applicant has amended Claims 13 and 14, and support may be found, for example, at page 1, line 19 through page 2, line 3; at page 4, lines 9-15; at page 6, lines 1-9; at page 7,

lines 11-14; at page 8, lines 3-22; at page 15, lines 12 through page 16, line 5; and in Figures 1, 4 and 5.

Applicant has amended Claims 17 and 18 for clarity. Support for the amendment is found, for example, at page 1, line 19 through page 2, line 3; at page 4, lines 9-15; at page 6, lines 1-9; at page 7, lines 11-14; at page 8, lines 13-21; at page 15, lines 12 through page 16, line 5; and in Figures 1, 4 and 5.

Applicant believes that no new matter has been added by any of these amendments, and the Examiner is respectfully requested to enter the amendments.

Response

In the response that follows, the Examiner's specific objections and rejections are reiterated in small bold indented print, followed by Applicant's response, which is identified by normal print.

35 U.S.C. § 103

The Examiner maintained the rejection of Claims 1-12 made in the Office Action mailed October 9, 1996. In that action, the Examiner rejected Claims 1-5 as unpatentable over Weighardt *et al.* The Examiner has maintained the rejection of Claims 1-5 based on the assertion that:

> **First, the argument of the response [of February 10, 1997] are directed to methods limitation because the response argues about simultaneous use of primers under one set of conditions. It is submitted that these arguments are not applicable to product claims, for which " intended use" limitations are not given weight...**
>
> **... Second,...the method of Weighardt *et al.* use a primer which is the same as the primer of the invention...**
>
> **...Third, the response argues that Weighardt *et al.* neither teach nor disclose any sequence that provides a high stringency binding site. Nor does Weighardt *et al.* teach or suggest compositions or characteristics that provide such site(s).**
>
> **In response to this, it is p[o]inted out that Weighardt *et al.* does recite "the entire primers in turn become new and unique high stringency recognition sites in the following PCR cycles" (see pg. 77).**

Applicant partially avoids the Examiner's objection by amendment of Claim 1.

Applicant respectfully traverses the rejection of Claims 1-5, because Weighardt *et al.* 1) failed to suggest and taught away from the claimed invention, 2) failed to suggest a modification of the prior art by which the claimed invention could be successfully practiced, and 3) failed to suggest all the limitations of the claimed invention.

To establish a prima facie case of obviousness, three criteria must be met. (MPEP 2143). First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one skilled in the art, to modify the reference or to combine reference teachings. Second there must be a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)(citing *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 [Fed. Cir. 1988]). Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Royka and Martin*, 490 F.2d 981, 180 USPQ 580, 583 (CCPA 1974).

1) Weighardt *et al.* failed to suggest the claimed invention and taught away from the claimed invention.

"Before the PTO may combine the disclosures of two or more prior art references in order to establish prima facie obviousness, there must be some suggestion for doing so, found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art." *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941, 1943-44 (Fed. Cir. 1992).

The claimed invention, as amended, is directed to a plurality of oligonucleotide DNA primers having a 5'-X domain comprising a "...*common* sequence that does *not* hybridize to...multiple target sequences, said common sequence *not comprising a restriction enzyme recognition site sequence*;" the primers are employed to simultaneously amplify the multiple target sequences in a multiplex polymerase chain reaction (PCR)(e.g., Claim 1; emphasis

added). Each of the claimed primers also has a 3'-Y domain comprising a unique sequence contained within or flanking one of said multiple target sequences or its complement (e.g., Claim 1). In addition, each of said primers is "capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during...[multiplex PCR]...amplification." This is so, despite the fact that "the melting temperature of a hybrid between at least one of said 3'-Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR." Thus, the oligonucleotide primers of the claimed invention possess unique utility in that they can be pooled together with a DNA sample in a single multiplex PCR reaction mixture, which then can be subjected to a uniform set of amplification cycling times and annealing temperatures for simultaneous amplification of multiple target DNA sequences.

On the other hand, Weighardt *et al.* taught away from the primers of the claimed invention. Weighardt *et al.* suggested "tailed primers...containing 15-20 nucleotides complementary to the recognition sites on the template DNA, tailed at their 5'-ends with an unrelated sequence of 10-15 nucleotides" (page 77, column 1, last paragraph); contrary to the Examiner's assertion, these were not the primers of the claimed invention. Instead, the oligonucleotide primers of Weighardt *et al.* comprised a 17- or 18-mer 5'-tail that included a recognition site sequence for one or another restriction endonuclease (e.g., Figure 2, caption, lines 6-8; and Figure 3, caption, lines 5-9). The significance of this difference should not be overlooked. For example, Weighardt *et al.* taught that "from an applicative point of view our procedure is very simple and requires no preliminary work when, as is often the case, primers are already *tailed with restriction enzyme sites* for subsequent cloning. (Page 79, second complete paragraph; emphasis added). But the procedure of Weighardt *et al.* was not a multiplex PCR procedure, and Weighardt *et al.* could choose a restriction site sequence known to be unrelated to the *single* target sequence of interest. (See, page 77, column 1, last paragraph). In a multiplex PCR, as encompassed by the claimed invention, a 5'-tail sequence

that would include a restriction enzyme recognition site sequence would likely hybridize non-specifically with at least some of multiple target sequences that contained a cognate restriction site. And since, as contemplated in the specification as filed (see, page 9, lines 1-2), parts of some target sequences of interest might not be fully known, extensive preliminary experimentation would be required to determine whether a given 5'-tail sequence could be used in any given multiplex PCR amplification. Similarly, hybridizations between the restriction enzyme recognition site sequence in the 5'-tail of the primers of Weighardt *et al.* and complementary sequences in non-target DNA sequences might occur. Hybridizations such as these could introduce artifacts into a multiplex amplification process. Therefore, the primers of Weighardt *et al.* would not have the utility of the claimed primers in multiplex PCR.

In contrast, the 5'-X domains of each of the primers of the claimed invention comprise "a common sequence that does not hybridize to...multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence." (Claim 1 and Claims 2-4 dependent therefrom). In Claims 2 and 5, the 5'-X domain is further limited to the oligonucleotide sequence of SEQ ID NO:64, a "universal primer sequence," that does not include a restriction enzyme recognition site sequence, unlike the primers of Weighardt *et al.* (*See*, page 8, lines 19-22). Therefore, contrary to the Examiner's assertion, the primers used in the method of Weighardt *et al.* are not the same as the primers of the claimed invention. Nothing in Weighardt *et al.* would have suggested a "universal primer sequence" as a 5'-X domain, and the primers with 5'-tail sequences comprising restriction enzyme recognition site sequences, taught by Weighardt *et al.*, would have taught away from the claimed invention.

Weighardt *et al.* did not suggest oligonucleotide primers capable of annealing specifically with their cognate target sequences under uniform high stringency conditions during multiplex PCR amplification. Although Weighardt *et al.* taught that a slight modification of their method allowed the addition of both of a pair of tailed primers at the beginning of a standard PCR reaction, primer extension was first performed at the annealing temperature (T_1) of oligo 1, the primer with the lowest annealing temperature, and was then

followed by five PCR cycles at the higher annealing temperature (T_2) for oligo 2. Then followed 35 cycles of high stringency PCR (annealing temperature, 72°C; pages 78-79, bridging paragraph):

> The first primer extension reaction at T_1 generates two types of extended molecules: one from oligo 1 annealed at high stringency (type A), and the other from oligo 2 (type B) annealed at low stringency...[I]n the next five cycles at T_2 only type B molecules can be amplified exponentially. On the contrary, type A molecules increase only linearly, because T_2 is not permissive for the annealing of oligo 1. The final 35 cycles at high stringency (annealing temperature 72°C) will select further for amplification of the products of the initial primer extension with oligo 1. (pages 78-79, bridging paragraph).

Contrary to the Examiner's assertion, it was only in reference to these initial primer extension products, not to the oligonucleotide tailed primers, that Weighardt *et al.* recited that "the entire primers in turn become new and unique high stringency recognition sites in the following PCR cycles." (Page 77, second column, lines 8-11). Further, Weighardt *et al.* taught that after the first annealing reactions at the lower temperature T_1, which enriched the reaction mixture for type A molecules, the type B molecules "can be contaminated by molecules derived from spurious primings of oligo 2" at the low stringency temperature condition. (Page 79, lines 5-7). Consequently, Weighardt *et al.* actually taught oligonucleotide tailed primer pairs that are *incapable* of annealing specifically with their cognate target sequences under *uniform* high stringency annealing conditions. And due to the differential annealing efficiencies of these primers, in a multiplex PCR, they would not have the contemplated utility of the primers of the claimed invention, because differential amplification and accumulation of non-specific amplification products, as described by Weighardt *et al.*, would be magnified in a reaction mixture containing multiple pairs of oligonucleotide primers and multiple target sequences.

Therefore, Weighardt *et al.* did not suggest the chimeric oligonucleotide DNA primers of the claimed invention, which are capable of serving "as high stringency recognition

sequences for subsequent rounds of amplification," regardless of the fact that "the melting temperature of a hybrid between at least one of said 3'Y domains and its complement, in the absence of other sequences, is different from the melting temperature of a hybrid between at least one other 3'-Y domain and its complement present in said multiplex PCR." (*See*, specification as filed, page 8, lines 7-8 and Claim 1). They failed to suggest primers "capable of annealing specifically with their cognate target sequences under uniform high stringency annealing conditions during multiplex PCR amplification," the use of which could be used to normalize the annealing efficiency of different primers and their cognate target sequences. (See, Claim 1 and specification as filed, page 8, lines 7-10).

2) Weighardt *et al.* failed to suggest a modification of the prior art by which the claimed invention could be successfully practiced.

There must be a reasonable expectation of success. Whether an invention was predictable or whether the Examiner's proposed modification or combination of the prior art has a reasonable expectation of success is determined at the time the invention was made. *Ex parte Erlich*, 3 USPQ2d 1011, 1016 (Bd. Pat. App. & Inter. 1986).

As discussed above, Weighardt *et al.* taught away from the primers of the claimed invention. Although the oligonucleotide primers of Weighardt *et al.* were tailed at their 5' ends with a sequence unrelated to the single target sequence in a standard PCR, the tail comprised a recognition site for one or another restriction endonuclease (e.g., Figure 2, caption, lines 6-8; and Figure 3, caption, lines 5-9). The fact that these paired 5'-tailed primers of Weighardt *et al.* continued to exhibit differential annealing efficiencies in standard PCR would have taught away from predicting that a plurality of oligonucleotide DNA primers comprising two domains could be produced that would be capable of annealing specifically with their cognate target sequences under *uniform* high stringency annealing conditions during multiplex PCR amplification. Based on the teachings of Weighardt *et al.*, and absent the hindsight provided by the disclosures of the subject specification, one of skill in the art would

not have predicted that chimeric oligonucleotide primers having a 5'-X domain comprising "a common sequence that does not hybridize to...multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence" would have this capability. Nor would the teachings of Weighardt *et al.* have led one of skill in the art to predict that primers having a 5'-X domain comprising the sequence SEQ ID NO:64, a "universal priming sequence," would be capable of annealing specifically with their cognate target sequences under uniform high stringency annealing conditions during multiplex PCR.

3) Weighardt *et al.* failed to suggest all the limitations of the claimed invention.

Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

As previously discussed, Weighardt *et al.*, failed to suggest a plurality of oligonucleotide DNA primers having a 5'domain, X, wherein each X domain comprises "a common sequence that does not hybridize to...*multiple* target sequences, said common sequence not comprising a restriction enzyme recognition site sequence"...and wherein each of said primers is "capable of annealing specifically with its cognate target sequence under uniform high stringency annealing conditions during...[multiplex PCR]...amplification." (e.g., Claims 1-4). The paired primers of Weighardt *et al.* had differential annealing efficiencies even in standard PCR. Furthermore, Weighardt *et al.* merely suggested primer pairs having 5'-tails with an "unrelated sequence of 10-15 nucleotides," which comprised one or another restriction enzyme recognition site sequence, and they failed to suggest oligonucleotide DNA primers having the limitation of an 5'-X domain where X comprises the sequence of SEQ ID NO:64 (e.g., Claims 2 and 5), a "universal primer sequence," which does not comprise a restriction enzyme recognition site sequence. Therefore, Weighardt *et al.* failed to suggest all the limitations of the claimed invention.

The Examiner has failed to establish a *prima facie* case of obviousness. The Examiner has improperly combined and modified references without any motivation or suggestion in the

references to do so, has ignored teaching in the references that would direct the Examiner away from the combination, ignored claim limitations not suggested in the prior art, and used improper hindsight in finding the invention obvious. Unless the Examiner has other specific knowledge of the prior art which the Examiner would like to offer as support, Applicant respectfully requests the Examiner to withdraw the rejection of Claims 1-5 on this ground.

35 U.S.C. § 103

The Examiner maintained the rejection of Claims 1-12 made in the Office Action mailed October 9, 1996. In that action, the Examiner rejected Claims 6-12 as unpatentable over Picci *et al.* in view of Weighardt *et al.* The Examiner has also applied this rejection to Claims 13-18. The Examiner based the rejection of these claims on the assertion that:

> **...Second, the response argues that the invention uses one set of cycling times and temperature despite different annealing temperatures, and Weighardt *et al.* do not teach that the primers can be pooled together and used under a single set of cycling times and temperatures in a multiplex polymerase chain reaction, and further that Picci *et al.* teach away from the invention in that individual primer pairs are added to a reaction sequentially.**
>
> **Weighardt *et al.* do suggest that the method can attain the same result in one step using a single set of primers (see pg. 79).**
>
> **Even though Picci *et al.* do teach that the method requires individual primer pairs added to a single reaction sequentially, the method of Weighardt *et al.* use a primer which is the same as the primer of the invention and Weighardt *et al.* do suggest that the method can attain the same results using one step.**
>
> **Third, the response argues that Weighardt *et al.* neither teach nor disclose any sequence that provides a high stringency binding site. Nor does Weighardt *et al.* teach or suggest compositions or characteristics that provide such site(s).**
>
> **In response to this, it is p[o]inted out that Weighardt *et al.* does recite "the entire primers in turn become new and unique high stringency recognition sites in the following PCR cycles" (see pg. 77). The PCR procedure with the tail primer improved the specificity of the reaction. The benefit of using Weighardt *et al.*'s primers having non-complementary 5'-end tails would have been expected in the multiplex PCR method of Picci *et al.***

Applicant partially avoids the Examiner's objection by an amendment further limiting Claim 14. Applicant respectfully traverses the rejection of Claims 6-18, because Picci *et al.*, combined with Weighardt *et al.*, 1) failed to suggest the claimed invention, taught away from the claimed invention, and failed to provide enabling technology for practicing the claimed invention, 2) failed to suggest a modification of the prior art by which the claimed invention

could be successfully practiced, and 3) failed to suggest all the limitations of the claimed invention.

To establish a prima facie case of obviousness, three criteria must be met. (MPEP 2143). First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one skilled in the art, to modify the reference or to combine reference teachings. Second there must be a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)(citing *In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 [Fed. Cir. 1988]). Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Royka and Martin*, 490 F.2d 981, 180 USPQ 580, 583 (CCPA 1974).

The examiner bears the burden of establishing a prima facie case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993); *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Only if this burden is met does the burden of coming forward with rebuttal argument or evidence shift to the applicant. *Rijckaert*, 9 F.3d at 1532, 28 USPQ2d at 1956. Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. *In re Bell*, 26 USPQ 2d 1529, 1531 (Fed. Cir. 1993). Also, the Court of Appeals for the Federal Circuit has defined an effective reference or combination of references, for the purposes of § 103 obviousness, as one that contains "detailed *enabling methodology* for practicing the claimed invention, a suggestion to modify the prior art to practice the claimed invention, and evidence suggesting it would be successful." *In re O'Farrell*, 7 USPQ2d 1673, 1680 (Federal Circuit 1988; emphasis added).

1) Picci *et al.* and Weighardt *et al.* failed to suggest the methods of the claimed invention, taught away from the claimed invention, and failed to provide enabling methodology for

practicing the claimed invention.

"Before the PTO may combine the disclosures of two or more prior art references in order to establish prima facie obviousness, there must be some suggestion for so doing, found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art." *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941, 1943-44 (Fed. Cir. 1992).

The multiplex PCR method taught by Picci *et al.*, combined with the oligonucleotide tailed primers of Weighardt *et al.*, would not have suggested the claimed method for simultaneous amplification of multiple DNA target sequences present in a DNA sample (Claim 6), nor for simultaneously amplifying and detecting (the presence of) multiple defined target sequences in a DNA sample (Claims 7-9 and 13), nor for high-throughput genetic screening (Claims 10-12), nor the method of screening to simultaneously amplify and detect multiple target sequences of interest in DNA (Claims 14-16). Neither would the combined references have suggested a plurality of amplified target sequences of interest detected according to the claimed methods (Claims 17-18).

Picci *et al.* did not suggest the methods of the claimed invention. The claimed method, employ the claimed chimeric oligonucleotide DNA primers, discussed above, e.g., in the step of contacting a DNA sample with a multiplicity of paired oligonucleotide primers having the structure 5'-XY-3', wherein...each X comprises the sequence...(SEQ ID NO:64) (Claim 6-13) or wherein each 5'-X domain comprises a common oligonucleotide that is neither complementary to nor specific for said multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence (Claims 14-16). Thus the methods of the claimed invention impose a uniformly high degree of specificity on the annealing reactions that occur between different primers present in the mixture and their cognate target sequences (page 8, lines 3-5), such that once optimal concentrations of each primer pair are determined individually (page 11, lines 8-9), there is no need to perform further experimental manipulations to optimize cycling conditions or the proportions of primer concentrations in the multiplex system in order to avoid amplification artifacts.

In contrast, Picci *et al.* taught a multiplex PCR method, using non-chimeric primers, that required the sequential addition of the individual oligonucleotide primer pairs into a single reaction, followed by testing to determine whether conditions needed to be modified to enable approximately equal amplification of the different target fragments. (Page 553, columns 1-2, first two sentences of bridging paragraph). Also, Picci *et al.* taught that in order to prevent the formation of unwanted bands, it was necessary to adjust the concentration of each primer used in the reaction to "an *unusual proportion* of primer pairs." (Page 553, column 2, lines 7-11; emphasis added). Thus, Picci *et al.* taught away from the claimed methods, which do not require extensive optimization of all cycling conditions and primer concentrations in a multiplex PCR system, and they do not suggest an enabling technology, i.e., chimeric primers, by which the claimed methods could be practiced.

As discussed above, Weighardt *et al.* not only failed to teach "a primer which is the same as the primer of the invention," as the Examiner asserted, but they taught away from the primers of the claimed invention. The oligonucleotide primers of Weighardt *et al.* were tailed at their 5' ends with a sequence unrelated to the single target sequence in a standard PCR, but the tail comprised a recognition site for one or another restriction endonuclease (e.g., Figure 2, caption, lines 6-8; and Figure 3, caption, lines 5-9). In a multiplex PCR, as encompassed by the methods of the claimed invention, a 5'-tail sequence that would include a restriction endonuclease recognition site sequence would likely hybridize non-specifically with at least some of multiple target sequences that contained a cognate restriction site sequence, parts of which target sequences might not be fully known. Similarly, hybridizations between the restriction recognition sequence in the 5'-tail of the primers of Weighardt *et al.* and complementary sequences in non-target DNA sequences present in a DNA sample might occur. Hybridizations such as these could introduce artifacts into a multiplex amplification process. Also, due to the differential annealing efficiencies of the primers taught by Weighardt *et al.*, in a multiplex PCR they would not have the contemplated utility of the primers of the claimed invention, because differential amplification and accumulation of non-

specific amplification products, as described by Weighardt *et al.*, would be magnified in a single reaction mixture with a multiplicity of paired oligonucleotide primers and multiple target sequences as in the claimed methods. In contrast, the 5'-X domains of each of the primers used in the method of the claimed invention comprise "a common oligonucleotide sequence that is neither complementary nor specific for said multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence" (Claims 14-16), or more specifically, the 5'-X domains comprise the common sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64; Claims 6-13), a "universal primer sequence." And each of the claimed oligonucleotide primers is useful in a multiplex PCR amplification, under the same/identical/ideal (reaction) conditions and cycling parameters (Claims 6-13; i.e., cycles of identical melting, reannealing, and extending temperatures and times, e.g., Claims 14-16) to form amplification products for each of...multiple defined target sequences primed with said oligonucleotides." (Claims 7-13). Since the chimeric oligonucleotide primers used in the claimed methods are not those taught by Weighardt *et al.*, and since Weighardt *et al.* failed to teach or provide enabling technology for chimeric primers that would be useful in the steps of the claimed methods, there would have been no motivation for one of skill in the art to combine the teachings of Weighardt *et al.* with the teachings of Picci *et al.*

2) Picci *et al.* and Weighardt *et al.* failed to suggest a modification of the prior art by which the claimed invention could be successfully practiced.

There must be a reasonable expectation of success. Whether an invention was predictable or whether the Examiner's proposed modification or combination of the prior art has a reasonable expectation of success is determined at the time the invention was made. *Ex parte Erlich*, 3 USPQ2d 1011, 1016 (Bd. Pat. App. & Inter. 1986).

Contrary to the Examiner's assertion, the "benefit" of using Weighardt *et al.*'s primers having non-complementary 5'-end tails would *not* have been expected in the multiplex PCR

method of Picci *et al.* by one of skill in the art at the time of the claimed invention.

As discussed above, Weighardt *et al.* taught away from the primers of the claimed invention. This is because the oligonucleotide primers of Weighardt *et al.* were tailed at their 5' ends with a sequence unrelated to the single target sequence in a standard PCR, but the 5'-tails comprised a recognition site for one or another restriction endonuclease (e.g., Figure 2, caption, lines 6-8; and Figure 3, caption, lines 5-9), a likely source of artifacts in a method employing multiplex PCR. The fact that these tailed primers of Weighardt *et al.* continued to exhibit differential annealing efficiencies in standard PCR would have taught away from predicting that a plurality of oligonucleotide DNA primers comprising two domains could be produced that would be useful in the claimed methods, e.g., a method for simultaneous amplification of multiple DNA target sequences..., which comprise the step of "performing multiple cycles of melting, reannealing, and DNA synthesis under identical reaction conditions and cycling parameters" (Claims 6-9), or the step of "subjecting a... [DNA] sample to multiple cycles...conducted under the same reaction conditions and cycling parameters" (Claims 10-13), or the step of "contacting said sample...under multiplex [PCR] conditions wherein coamplification...occurs in one or more cycles of identical melting, annealing and extending temperatures and times" (Claims 14-16). Based on the teachings of Weighardt *et al.*, and absent the hindsight provided by the disclosures of the subject specification, one of skill in the art would not have predicted the usefulness in practicing the claimed methods of chimeric oligonucleotide primers having a 5'-X domain comprising "a common oligonucleotide sequence that is neither complementary to nor specific for said multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence" (Claims 14-16). One of skill in the art also would not have predicted successfully practicing the claimed methods using primers having 5'-X domains comprising the sequence 5'-GCGGTCCCAAAAGGGTCAGT-3' (SEQ ID NO:64; Claims 6-13), without undue experimentation. And without the claimed methods, one of skill in the art could not have predictably produced the "products-by-process" of a plurality of amplified target sequences of

interest amplified and detected according to the methods (Claims 17 and 18).

3) Picci *et al.* and Weighardt *et al.* failed to suggest all the limitations of the claimed invention.

Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

Picci *et al.* and Weighardt *et al.* failed to suggest all the limitations of the claimed methods. As discussed above, Picci *et al.* and Weighardt *et al.* failed to suggest all the limitations of the step of contacting a DNA sample with a multiplicity of paired "oligonucleotide primers having the structure 5'-XY-3', wherein...each X comprises the sequence...(SEQ ID NO:64)" (Claim 6-13) or wherein each 5'-X domain comprises "a common oligonucleotide that is neither complementary to nor specific for said multiple target sequences, said common sequence not comprising a restriction enzyme recognition site sequence" (Claims 14-16).

As discussed above, the paired primers of Weighardt *et al.* had differential annealing efficiencies in a standard PCR method. Weighardt *et al.* taught that a slight modification of their method allowed the addition of both of a pair of tailed primers at the beginning of a standard PCR reaction, but primer extension was first performed at the annealing temperature (T_1) of oligo 1, the primer with the lowest annealing temperature, and was then followed by five PCR cycles at the higher annealing temperature (T_2) for oligo 2. Then followed 35 cycles of high stringency PCR. This would not have suggested all the limitations of the step of "performing multiple cycles of melting, reannealing, and DNA synthesis under *identical* reaction conditions and cycling parameters" (Claims 6-9), nor all the limitations of the step of "subjecting said [DNA] sample to multiple cycles of melting, reannealing, and DNA synthesis wherein each of said cycles is conducted under the *same* (reaction) conditions and cycling parameters to form amplification products" (Claims 10-13), nor all the limitations of the step of "contacting said sample...under multiplex [PCR] conditions wherein

coamplification...occurs in one or more cycles of *identical* melting, annealing and extending temperatures and times" (Claims 14-16). (emphasis added).

Since Picci *et al.* and Weighardt *et al.* failed to suggest all the limitations of the claimed methods, they also failed to suggest all the limitations of the products, "a plurality of amplified target sequences of interest" amplified and detected according to the method of Claim 13, or Claim 14, respectively (Claims 17 and 18).

The Examiner has failed to establish a *prima facie* case of obviousness. The Examiner has improperly combined and modified references without any motivation or suggestion in the references to do so, has ignored teaching in the references that would direct the Examiner away from the combination, ignored claim limitations not suggested in the prior art, and used improper hindsight in finding the invention obvious. Unless the Examiner has other specific knowledge of the prior art which the Examiner would like to offer as support, Applicant respectfully requests the Examiner to withdraw the rejection of Claims 6-18 on this ground.

35 U.S.C. § 132

The amendment filed February 10, 1997 has been objected to under 35 U.S.C. 132, because it purportedly introduces new matter into the disclosure of the invention. The Examiner has based the objection on the assertion that there is no support for "a plurality of amplified target sequences of interest" in the specification.

The Applicant respectfully traverses this rejection, because support for "a plurality of amplified target sequences of interest" can be found in the specification as filed, for example, at page 1, line 19 through page 2, line 3; at page 4, lines 9-15; at page 6, lines 1-9; at page 7, lines 11-14; at page 8, lines 13-21; at page 9, lines 1-5; at page 15, lines 12 through page 16, line 5; and in Figures 1, 4 and 5.

"...The claims must find clear support or antecedent basis in the description so that the meaning of the terms in the claims may be ascertainable by reference to the description." 37

C.F.R. § 1.75(d)(1). The disclosures of the specification as filed identify "a plurality of amplified target sequences of interest" with "amplification products" that were amplified, isolated and detected by the claimed methods (using the claimed chimeric primers in a multiplex PCR and, e.g., gel electrophoresis), so that the meaning of the term is ascertainable by reference to the specification's description. The specification discloses that "...PCR has gained widespread use for the diagnosis of inherited disorders and susceptibility to disease. Typically, the *genomic region of interest is amplified* from either genomic DNA or from a source of specific cDNA encoding the cognate gene product." (Page 1, line 19 through page 2, line 3; emphasis added). The specification discloses that "the present invention encompasses methods and compositions that allow the efficient and essentially simultaneous *amplification* of different *target sequences* in a single polymerase chain reaction (i.e., multiplex PCR)" (page 7, lines 11-12; emphasis added). Further disclosed is that "the invention encompasses a method for detecting multiple defined *target DNA sequences* in a DNA sample" (page 4, lines 9-10; emphasis added), and that "this method includes a further step of detecting the *amplification products*, preferably by gel electrophoresis. In this embodiment, the presence or absence of an *amplification product* is diagnostic of the presence or absence of the target sequence in the original DNA sample." (Page 4, lines 12-15; emphasis added). The specification describes how a DNA sample was subjected to multiplex PCR amplification according to the claimed method, using primer sets specific for several (i.e., *a plurality* of) target sequences of interest, including the CFTR locus, α-galactosidase gene, sickle-cell gene, Tay-Sachs gene, β-thalassemia and WT-1 genes. (Page 6, lines 1-9; page 15, lines 10-18). Some sequences of interest are described in scientific literature cited in the specification (e.g., page 15, lines 13-17), and many target sequences of interest are defined in Figure 1 (e.g., SEQ ID NOS:1-63).

The specification discloses that "multiplex PCR according to present invention utilizes chimeric oligonucleotide primers that include two domains. The 5' 'half' of each primer may comprise any sequence between 17 and 25 bases in length that is unrelated to the target DNA,

and has the property of forming hybrids with relatively high melting temperatures." (Page 8, lines 13-16). "The 3' 'half' of each primer comprises a target-specific sequence, i.e., a sequence that is either present or potentially present in the target DNA or its complement. These 3' sequences may comprise without limitation any such sequence of 17-25 bases...irrespective of the melting temperature of hybrids formed between the isolated sequence and its complement." (Page 9, lines 1-5). "In a preferred embodiment, the 5' sequence comprises...a 'universal primer sequence' (UPS)..." (Page 8 lines 19-21). The specification discloses that for the multiplex PCR reactions, the resulting banding patterns of which are displayed in Figures 4 and 5, the chimeric ("UPS-tagged") primer pairs of the claimed invention "generate only the desired bands" in gel electrophoresis (page 15, lines 10-19). And the specification discloses that "for 13 of 14 UPS-tagged primer pairs, the expected bands are clearly prominent and virtually free of spurious *amplification products*." (Page 16, lines 1-2; emphasis added).

These disclosures identify "a plurality of amplified target sequences of interest" with "amplification products" that were amplified and detected by the claimed methods, so that the meaning of the term is ascertainable by reference to the specification's description. Because the disclosures in the specification as filed provide ample support for "a plurality of amplified target sequences of interest," Applicant believes that no new matter is added by the term. Therefore, the Examiner is respectfully requested to withdraw the objection to the amendment of February 10, 1997, on this ground.

35 U.S.C. § 112, first paragraph.

Claims 17 and 18 are rejected under 35 U.S.C. 112, first paragraph, as failing to provide an enabling disclosure. The Examiner bases this rejection on the assertion that these claims:

> **contain[ ] subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. A plurality of amplified target sequences of interest was not described in the specification.**

This rejection is respectfully traversed, because the specification as filed is enabling for and describes "a plurality of amplified target sequences of interest." Although working examples are not required of an enabling specification, here the specification has provided them.

Compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, does not turn on whether an example is disclosed (*See*, MPEP 2164.02). The specification need not contain a working example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation. *In re Borkowski*, 422 F.2d 904, 164 USPQ 642, 645 (CCPA 1970). The test of enablement is whether one skilled in the art could make or use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988).

Claims 17 and 18 are directed to "a plurality of amplified target sequences of interest amplified and detected according to the method" of Claim 13, or Claim 14, respectively (Claims 17 and 18). As discussed above, there is support for "a plurality of amplified target sequences of interest" in the specification. The specification identifies "a plurality of amplified target sequences of interest" with "amplification products" that were amplified, isolated and detected by the claimed methods (using multiplex PCR and, e.g., gel electrophoresis), such that the meaning of the term is ascertainable by reference to the specification's description. And the specification is enabling for the claimed invention. The specification discloses that "PCR has gained widespread use for the diagnosis of inherited disorders and susceptibility to disease. Typically, the *genomic region of interest is amplified* from either genomic DNA or from a source of specific cDNA encoding the cognate gene product." (Page 1, line 19 through page 2, line 3; emphasis added). The specification discloses that "the present invention encompasses methods and compositions that allow the efficient and essentially simultaneous *amplification* of different *target sequences* in a single

polymerase chain reaction (i.e., multiplex PCR)." (Page 7, lines 11-12; emphasis added). The specification discloses that "multiplex PCR according to present invention utilizes chimeric oligonucleotide primers that include two domains. The 5' 'half' of each primer may comprise any sequence between 17 and 25 bases in length that is unrelated to the target DNA, and has the property of forming hybrids with relatively high melting temperatures (e.g., T_ms > 60°C in the absence of other sequences)." (Page 8, lines 13-16). The specification discloses that "in a preferred embodiment, the 5' sequence comprises 5'-GCGGTCCCAAAAGGGTCAGT-3', a 'universal primer sequence' (UPS)..." and discloses its derivation from the phage vector M13mp18. (Page 8 lines 19-22). "The 3' 'half' of each primer comprises a target-specific sequence, i.e., a sequence that is either present or potentially present in the target DNA or its complement. These 3' sequences may comprise without limitation any such sequence of 17-25 bases...irrespective of the melting temperature of hybrids formed between the isolated sequence and its complement." (Page 9, lines 1-5).

The specification discloses that in practicing the claimed invention, a DNA sample is contacted with pairs of chimeric oligonucleotide primers under conditions suitable for polymerase chain reaction and specifies some well known PCR reaction conditions that may be used. (Page 11, lines 1-6). Ranges for primer concentrations and annealing temperatures are disclosed, as are precise cycling conditions. (Page 11, lines 7-14; page 13, lines 5-12). The specification teaches "that any DNA sample may be used in practicing the present invention, including eukaryotic, prokaryotic and viral DNA" (page 10, lines 8-9),...extracted from the cell source or body fluid using any of the numerous methods that are standard in the art," depending on the nature of the source, and discloses the remarkable sensitivity of the amplification process, i.e., that "the preferred amount of DNA to be extracted for use in the...invention is at least 5 pg (corresponding to about 1 cell equivalent of a genome size of 4 x 10^9 base pairs)." (Page 10, lines 14-19). The specification teaches that "equivalent banding patterns are observed over an 8-fold range of template concentrations when the UPS-tagged primer pairs are employed," in contrast to the template concentration-dependence evidenced

when standard primers are used. (Page 15, lines 2-6; Figure 3).

Further disclosed is that "the invention encompasses a method for detecting multiple defined *target DNA sequences* in a DNA sample" (page 4, lines 9-10; emphasis added), and that "this method includes a further step of detecting the *amplification products*, preferably by gel electrophoresis, a technique well-known in the art. In this embodiment, the presence or absence of an *amplification product* is diagnostic of the presence or absence of the *target sequence* in the original DNA sample." (Page 4, lines 12-15; emphasis added).

By way of exemplification, the specification describes how a DNA sample was subjected to multiplex PCR amplification according to the claimed method, using primer sets specific for several (i.e., *a plurality* of) target sequences of interest, including the CFTR locus, α-galactosidase gene, sickle-cell gene, Tay-Sachs gene, β-thalassemia and WT-1 genes (page 6, lines 1-9; page 15, line`s 10-18). Also, some sequences of interest are described in the art cited in the specification (e.g., page 15, lines 13-17), and many target sequences of interest are defined in Figure 1 (e.g., SEQ ID NOS:1-63). The specification discloses that for the multiplex PCR reactions, the resulting banding patterns of which are displayed in Figures 4 and 5, the chimeric ("UPS-tagged") primer pairs of the claimed invention "generate only the desired bands" in gel electrophoresis (page 15, lines 10-19). And the specification discloses that "for 13 of 14 UPS-tagged primer pairs, the expected bands are clearly prominent and virtually free of spurious amplification products." (Page 16, lines 1-2).

These disclosures would indeed convey to one of skill in the art that Applicant "had possession of the invention" at the time the application was filed. But more importantly, based on the disclosures in the specification as filed, one of skill in the art could practice the claimed invention without undue experimentation and thus obtain the claimed product of "a plurality of amplified target sequences of interest amplified and detected according to the method" of Claim 13 or 14 (Claims 17 and 18, respectively).

Since the Examiner has failed to cite references that provide a reasonable basis to question the enablement disclosed in the specification, the Examiner has failed to meet the

burden of providing sufficient reasons to doubt the assertions in the specification. Unless the Examiner has other specific knowledge of the prior art which the Examiner would like to offer as support, Applicant respectfully requests the Examiner to withdraw the rejection of Claims 17 and 18 on this ground.

35 U.S.C. § 112, second paragraph.

The Examiner rejected Claims 17 and 18 under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner bases this rejection on the assertion that:

> **These claims are made confusing by the language "a plurality of amplified target sequences of interest." It cannot be determined from the specification what is encompassed by a plurality of nucleic acids defined only by how said nucleic acids are detected.**

The rejection is partially avoided by the amendments of Claims 13, 14, 17 and 18. Applicant respectfully traverses the rejection of Claims 17 and 18, because the specification describes what is encompassed by "a plurality of amplified target sequences of interest amplified and detected" according to the claimed methods, and product-by-process claims are permissible.

A product-by-process claim, which is a product claim that defines the claimed product in terms of the process by which it is made, is proper. *In re Luck*, 177 USPQ 523 (CCPA 1973).

Claims 17 and 18 are directed to "a plurality of amplified target sequences of interest amplified and detected according to the method" of Claim 13, or Claim 14, respectively (Claims 17 and 18). As discussed above, there is support for "a plurality of amplified target sequences of interest" in the specification. The specification identifies the term "a plurality of amplified target sequences of interest" with "amplification products" that were amplified and detected by the claimed methods (using the previously discussed claimed chimeric primers in a multiplex PCR and, e.g., gel electrophoresis), so that the meaning of the term is ascertainable by reference to the specification's description. The specification discloses that "the present invention encompasses methods and compositions that allow the efficient and essentially

simultaneous *amplification* of different *target sequences* in a single polymerase chain reaction (i.e., multiplex PCR)" (page 7, lines 11-12; emphasis added), e.g., as in "a method of screening to simultaneously amplify and detect multiple target sequences", as in Claim 14. "Typically, the *genomic region of interest is amplified* from either genomic DNA or from a source of specific cDNA encoding the cognate gene product." (Page 1, line 19 through page 2, line 3; emphasis added). The specification teaches "that any DNA sample may be used in practicing the present invention, including eukaryotic, prokaryotic and viral DNA" (page 10, lines 8-9),...extracted from the cell source or body fluid using any of the numerous methods that are standard in the art," depending on the nature of the source, and discloses that "the preferred amount of DNA to be extracted for use in the...invention is at least 5 pg (corresponding to about 1 cell equivalent of a genome size of 4×10^9 base pairs)." (Page 10, lines 14-19). Further disclosed is that "the invention encompasses a method for detecting multiple *defined target DNA sequences* in a DNA sample" (as in Claim 13; page 4, lines 9-10; emphasis added), and that "this method includes a further step of detecting the *amplification products*, as in both Claims 13 and 14, preferably by gel electrophoresis. By way of exemplification, the specification describes how a DNA sample was subjected to multiplex PCR amplification according to the claimed method, using primer sets specific for several (*a plurality* of) target sequences of interest, including the CFTR locus, α-galactosidase gene, sickle-cell gene, Tay-Sachs gene, β-thalassemia and WT-1 genes (page 6, lines 1-9; page 15, lines 10-18). Also, some sequences of interest are described in the art cited in the specification (e.g., page 15, lines 13-17), and many target sequences of interest are defined in Figure 1 (e.g., SEQ ID NOS:1-63).

From these disclosures of the specification as filed, it would be clear to one of skill in the art that "a plurality of amplified target sequences" in Claims 17 and 18 encompass DNA sequences "amplified and detected" according to the steps of the claimed methods. As detailed in previous sections, the specification describes the steps of the methods or processes encompassed by Claims 13 and 14, including "...simultaneously contacting said [DNA] sample

with a plurality of oligonucleotide pairs, etc.,...subjecting said [DNA] sample to multiple cycles of melting, reannealing, and DNA synthesis...to form amplification products for each of said multiple defined target DNA sequences...; and detecting the amplification products" (Claim 13), or "...contacting said [DNA] sample with a plurality of oligonucleotide primer pairs having the structure, etc.,...under multiplex polymerase chain reaction conditions wherein coamplification of said multiple target sequences occurs...; and detecting the amplification products" (Claim 14). One of skill in the art would readily be able to distinguish "a plurality of amplified target sequences" that are products of the of the claimed methods, or processes, from other products that are not "amplified and detected" according to the methods of Claim 13 or 14. Therefore, since product-by-process claims are permissible, Applicant respectfully requests the Examiner to withdraw the rejection of Claims 17 and 18 on this ground.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

Respectfully submitted,

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